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STUDY OF AFRICAN TRYPANOSOMIASIS

Annual Report  
(1 October 1978-30 September 1979)

I. Muriithi

September 30, 1979

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Studies of natural and acquired resistance of bovines to <u>Trypanosoma</u> <u>congolense</u> showed that age resistance was substantial. Most (91%) of bovines under 1 year old survived infection without treatment, while only 11% of the; animals between 1 and 2 years old and none of those over 2 years old survived without treatment. Specific maternal antibody was not involved, since all calves were from non-infected dams and had been weaned at least 1 month prior to infection. Animals that self-cured remained resistant for extended periods			

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Survival times of  $^{51}\text{Cr}$ -labeled thrombocytes were compared in I. congolense infected and normal steers. Average thrombocyte survival was only 1.3 ( $\pm 0.5$ ) days in infected bovines compared to 3.7 ( $\pm 0.5$ ) days in uninfected controls. This indicates that the thrombocytopenia observed in I. congolense infected cattle results from an increased rate of thrombocyte destruction rather than a reduction or inhibition of thrombocyte production. The mechanism of thrombocyte destruction associated with thrombocytopenia in I. congolense infected cattle remains to be defined.

Average weight of G. morsitans pupae from laboratory reared adults fed exclusively on live bovine hosts was 27.9 mg (n = 2,830), and adult emergence from these pupae averaged 90%. Young flies invariably fed to repletion at each feeding interval (6 interval per week) but the feeding rate dropped to 90.2% per interval among older flies. Pregnant female flies just prior to larviposition often failed to feed during 15 min exposures on a bovine hosts, indicating that such flies should not be used in transmission studies. Dissections of 85 male and 111 female G. morsitans after both sexes were exposed to I. congolense on the same infected steer showed that males had higher rates of cyclic infection than females. Repeated feedings on infected hosts resulted in slightly higher infection rates in both sexes than did the teneral (initial) feeding alone.

The culture of Leishmania donovani in Schneider's insect medium was evaluated as a means of diagnosis and management of patients with visceral leishmaniasis. Samples from 13 patients examined prior to treatment showed that smears of splenic aspirate material and parallel cultures from the same samples were all positive. The techniques were compared 68 times during subsequent therapy, and in 8 instances cultures proved positive while no parasites were detected on the smears. Most cultures were positive within 3-4 days. The culture technique appears more sensitive than stained smear examination and thus provides a better method of assessing the effectiveness of drug therapy.

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TRYPANOSOMA CONGOLENSIS: NATURAL AND ACQUIRED RESISTANCE  
IN THE BOVINE

INTRODUCTION

In nature, there is some evidence of natural and acquired immunity in cattle to trypanosomiasis. It has been postulated that young animals are more resistant to trypanosomiasis than adults (Reviewed by Fiennes, 1970), possibly through transmission of an immune factor to calves born of immune or partially immune dams (Whiteside, 1962). Certain breeds of cattle also appear to be naturally resistant to trypanosome infections (Stephen, 1970). Attempts to induce immunity to trypanosomiasis under field conditions, however, have produced contradictory results. Reports of several investigators have shown no evidence of immunity in cattle maintained under therapy in endemic areas over long periods of time (Hornby, 1941; Wilson, Paris and Dar, 1975). Other workers, however, claim that drug therapy induced a degree of protective immunity in treated animals (Bevan, 1928; van Saceghem, 1938; Fiennes, 1953; Soltys, 1955; Smith, 1958; Wilson, et al. 1976). Many of these field observations, however, are difficult to interpret because of the use of small numbers of animals of unknown age and condition, the question of persisting trypanocides, and the meager information concerning the antigenic nature of the trypanosome complex in given endemic areas.

In the laboratory on the other hand, a variety of immunization procedures have been used which produce a strong resistance in animals to a challenging trypanosome infection (Dodin and Fromentin, 1962; Johnson, et al. 1963; Seed and Weinman, 1963; Duxbury and Sadun, 1969; Wellde, et al. 1979). None of these procedures, however, has been shown to be effective against the disease in nature. This has been due, in part, to the variant specific nature of the protective immune response and to the relatively obscure antigenic structure of the naturally transmitted metacyclic trypanosome. The early literature on the subject of immunity to African trypanosomiasis has been amply reviewed by Taliaferro (1929), and the more recent literature by Clarkson (1976) and Murray and Urquart, (1977).

The lack of substantial laboratory investigations regarding immunity in the bovine to Trypanosoma congolense led us to examine the questions of immunity in reference to the following: age resistance, self-cure, chemotherapeutic cure and the relationship between blood and tsetse fly induced infections.

MATERIALS AND METHODS

Animals

Cattle of a predominantly Hereford breed were obtained from the veterinary department farm at Kabete or from other trypanosomiasis-

free areas in Kenya. Upon their arrival at our laboratory, all animals were routinely treated before experimentation with recommended levels of Terramycin (Pfizer International - New York, New York), Phenamidine (May and Baker, Dagenham, England) and Ranizole (Merck Sharpe and Dohme, B.V., Haarlem, Netherlands). Ranizole treatment was continued on a periodic basis. All animals also received foot and mouth vaccine (Wellcome-Kenya). In general, the experimental animals were kept outside and supplemental food was provided during periods of poor pasture conditions. All experimental animals were dipped or sprayed in an acaricide weekly, with the exception of animals undergoing tsetse fly challenge.

#### Trypanosomes

The Trans-Mara I strain of Trypanosoma congolense which was isolated from an infected cow in the Trans-Mara area near the Kenya-Tanzania border in 1966 was the primary parasite used in these studies. A stabilate was made from a pool of blood collected from 3 infected steers in 1971. Other stabilates were prepared in 1973 and 1975 and all animals in this study were infected with trypanosomes originating from one of these three stabilates. Usually, infected mice were used as donors after being infected with stabilate trypanosomes. Sometimes, however, animals were infected or challenged with blood obtained from infected cattle.

For blood induced infection or challenge, trypanosomes in heparinized blood were enumerated in a hemocytometer and diluted with phosphate buffered saline (pH 7.8) containing 5% glucose and 10% fetal calf serum and injected into the jugular vein. Cattle were infected and challenged with 10,000 Trypanosoma congolense per 500 Lbs. body weight unless otherwise noted.

For tsetse fly infection or challenge, newly emerged flies (Glossina morsitans) were fed on an infected bovine donor for 14 consecutive days. Thereafter, the flies were fed for 5-day intervals on non-infected bovines until needed to induce a challenging infection.

A second strain of T. congolense was used for testing immunity to a heterologous strain. This parasite (designated Yoani I strain) was isolated in 1977 from an infected dairy cow at Yoani, Kenya, about 40 miles south of Nairobi.

#### Detection of Parasites

All animals were tested for the presence of trypanosomes by injecting their blood (0.5 ml) into mice intraperitoneally before the initiation of experiments. Subinoculations of blood were also done in some experimental animals in an effort to detect subpatent infections.

Parasitemias in experimental animals were estimated by counting the numbers of trypanosomes per 100 leucocytes on thick blood smears and relating these values to the total leucocyte counts per cubic millimeter.

### Chemotherapy

Curative chemotherapy was initiated with Berenil (Farbwerke Hoechst, Frankfurt (M) Germany) at a level of 1.05 g of active ingredient per 660 lbs. of body weight. Generally, animals which were treated were severely anemic, extremely weak and occasionally prostrate. These animals appeared to be near death at the time of treatment.

### Assessment of Immunity

Immunity in experimental animals was assessed by comparing prepatent periods, levels and frequency of parasitemia, hematologic parameters, general clinical signs, and the ability to survive a challenging infection with those of controls.

### Hematology

Packed cell volumes (PCV) were done by the microhematocrit method and leucocytes were counted using an electronic cell counter (Coulter Electronic, Harpenden, England). Methods used in collecting samples and counting thrombocytes have been published previously (Wellde *et al.* 1978). Reference in the text to experimental values are given plus or minus one standard deviation ( $\pm$ SD) unless otherwise noted.

## RESULTS

### Effect of dose of trypanosomes and sex of host

Within the range of numbers of trypanosomes injected into cattle, no relationship between dose and the survival time of animals was observed. The dose of trypanosomes was, however, related inversely to the prepatent period (Fig. 1). Both male and female animals developed similar infections and there was no apparent difference in survival times between animals of different sexes.

### Age Resistance

Table 1 depicts the results of infections in animals of different age groups. It can readily be seen that most animals under one year of age survived the infection without treatment. Some animals between 1 and 2 years of age also were able to survive the infection, whereas all animals over 2 years of age either died or required treatment to survive.

### Infections in Young Animals

Eventhough young animals were able to survive the infection without treatment, they underwent a severe disease. Clinical parameters were compared in 11 young animals who survived and 6 uninfected controls over a 31-week period. Fig. 2 shows the average level of trypanosomes in the peripheral blood of the survivors over a 31-week period. Average levels



of parasitemia were gradually reduced as the disease progressed. While animals had patent infections throughout the first 8 weeks, after this time an increasing number of animals became apatent for periods which became greater with time. An average preinfection packed cell volume of  $34.0(\pm 4.0)$  was reduced to a level of  $17.9(\pm 3.2)$  at 8 weeks after infection (Fig. 2). Packed cell volumes gradually increased after this time and by 31 weeks after infection had risen to  $24.1(\pm 4.8)$ . Packed cell volumes did not appear to reach preinfection levels in individual animals for long periods even though trypanosomes were only infrequently found in the blood. Thrombocytopenia and leucopenia were also prominent manifestations of the disease (Fig. 2). Intermittent fever was accompanied by an early weight loss after which a minimal weight gain was apparent. Controls of the same age, however, had gained an average of 116 Lbs. while the infected animals gained only an average of 9 Lbs. during the 31-week period (Fig. 2). Many of these young infected animals remained small in stature throughout their adult life (Fig. 3).

#### Immunity in Self Cured Calves

Animals which had apparently self cured the primary infection and whose blood was negative when subinoculated into mice were challenged with the same strain of Trypanosoma congolense up to a year after their last patent parasitemia. No detectable infections developed in the self cured animals whereas the controls developed typical infections and required treatment to survive (Table 2).

#### Infections in Adult Animals

Animals over one year of age developed an acute or chronic course of disease that was usually considered fatal. Clinical parameters were compared in 11 adults and 11 uninfected controls (Fig. 4). The average parasitemia in adult animals was twice that of the surviving younger animals, however, the anemia which developed was similar in degree. A leucopenia which was comparable to that found in young animals was also present (Fig. 4). Average thrombocyte levels were lower in infected adult animals although younger animals naturally have a higher level of thrombocytes. Weight loss was marked in adult animals with up to a 34% decrease in preinfection values. Ten of the 11 infected animals died or required treatment to survive by the 15th week of infection. The remaining infected animal developed a protracted chronic course of disease and died during the 32nd week of infection. This chronic disease state was characterized by a low level relapsing parasitemia accompanying a continued low PCV.

#### Immunity in Treated Adults

Adult animals which required therapy to survive were challenged along with controls at a later time. Table 3 shows that an appreciable immunity had developed in these animals and many self cured the challenge infection. Even when the challenging infection was given about two years after treatment there was evidence of persisting immunity. Although most of these animals which were challenged at this time needed treatment to survive, their infections were of a chronic nature and less severe than

those of control animals. Treatment was required in these animals at a later time than in their primary infections or in the controls. Figure 5 illustrates the pattern of parasitemia and level of packed cell volume in an animal undergoing infection, treatment and challenge. The challenging infection was much less severe than the primary infection; the animal had limited periods of parasitemia which were similar to that of a chronically infected animal and a minimal decrease in packed cell volume and other hematologic parameters.

When animals described in Table 3 were challenged a second time, no detectable infections or clinical signs of disease were observed while all controls developed parasitemia and required treatment to survive (Table 4). Animals were also strongly resistant to challenge with relapse parasites obtained from chronically infected bovines. Figure 6 illustrates the patterns of parasitemia and levels of packed cell volumes in an animal immunized by infection and cure and a control animal challenged with parasites isolated from a bovine undergoing a relapsing infection of 250 days duration. When compared to the control animal, the infection in the immunized animal was brief and much less severe. The control required treatment while the immunized animal self cured.

#### Tsetse Fly Challenge

Animals presumed to be immune to challenge with blood forms were subjected to tsetse fly challenge with the homologous strain of Trypanosoma congolense. Each of twelve immune, three partially immune and nine control animals received an average of 428 fly bites from a pool of flies having a 32% infection rate of metacyclic trypanosomes. Of the twelve immune bovines challenged by fly bite, five did not develop parasitemia or clinical evidence of disease (Table 5). The other seven had limited periods of patent parasitemia (Fig. 7), and only one animal developed signs of clinical disease. All twelve immune animals survived without treatment while all nine control animals developed severe infections and eight required treatment to survive.

Average parasitemias were greatly reduced in immune animals and followed a relapsing pattern somewhat similar to that of chronic infections or that of immune animals challenged with blood forms. Prepatent periods were not always increased in immune animals, however, and three immune animals had prepatent periods similar or shorter than controls. Although parasites appeared in the blood of these animals early after challenge they were suppressed quickly (Fig. 8). Clinical parameters such as PCV (Fig. 9) thrombocyte levels (Fig. 10) and leucocyte levels (Fig. 11) remained within normal limits in immunized animals while the values in controls were severely affected.

One year after cyclical transmission was initiated, experimental animals remained highly resistant to challenge with the Trans Mara strain by fly bite whereas control animals required treatment to survive.

One animal, which had undergone primary infection and challenge in 1970 and was rechallenged periodically during the subsequent 6 years with syringe induced infections, was challenged by tsetse fly bite in 1977. Table 6 summarizes the results over the 8-year period. Control animals injected at each challenge either required treatment to survive or died.

#### Heterologous Challenge

To determine whether or not cross strain immunity was present in animals immune to the Trans Mara I strain of T. congolense, 3 immune and 3 control animals were challenged with the Yoani strain of T. congolense by blood induced or tsetse fly induced infections (Table 7). No immunity was observed in any of the animals whether challenged by either method. All animals were treated during the fifth week of infection when packed cell volumes had decreased to below 20%.

#### DISCUSSION

Our studies demonstrate that under certain conditions an appreciable immunity to T. congolense can develop in bovines. We found a substantial age resistance to Trypanosoma congolense and although young animals underwent a relatively severe disease process, almost all survived while animals over two years of age invariably succumbed to infection. Although the mechanism(s) for such resistance is not clear, in our experiments it did not involve specific maternal antibody since the dams of our calves had never been infected and the calves had been weaned at least 1 month before infection. These studies confirm and extend the observations of others (reviewed by Fiennes, 1970). Although a more virulent strain of T. congolense might kill young animals, we believe a relative resistance would be found in them when compared to adults.

Although Weitz (1970) suggested there was no evidence for an acquired protection in animals after recovery from the disease, we have shown that young surviving animals are resistant for extended periods of time to a challenge infection of the same strain by either syringe inoculation of blood forms or by tsetse fly bite (metacyclic forms). Many of these immune animals, however, are stunted and are relatively non productive. As well as being a poor source of meat, the small stature of females infected early in life may lead to problems in calving. We have observed the death of one of our self-cured experimental animals due to the inability to complete parturition because of her small pelvic diameter.

Animals undergoing infection and Berenil treatment also showed resistance upon syringe or tsetse challenge with the same strain. Most of these animals self cured the first or second challenge infections. Premunity did not play a role in this protection since the animals had been given curative therapy to terminate the primary infection. The resistance appeared to be associated with the duration of infection, the

time elapsing between treatment and challenge and the number of infections the animal has been subjected to. The short period that effective levels of Berenil persist in the blood of the bovine precludes any complicating drug effect in these studies. Trials in our laboratory showed that Berenil (7 mg/kilo) had an effect on infectivity for 12 days and on the prepatent period for up to 18 days but not at 25 days or longer after injection (Unpublished data). This is in agreement with previously published work (Cunningham et al. 1964).

While we have shown that a substantial immunity can be induced experimentally by infection and cure, the reasons are not well understood why it has not been more apparent in nature. Since most of the failures to produce an immunity in animals in the field by this method have been in areas of high tsetse challenge, we believe that the interval between treatment and reinfection is important. It is known that the lymphoid system in T. congolense infected bovines undergoes atrophy and depletion of lymphocytes (Kalinin, 1974), (Murray, 1974). Morrison and Murray (1979) have shown a marked depletion of immunoglobulin containing cells in the spleens of T. congolense infected mice and these findings are consistent with the reports of deficient immunologic responses to a variety of antigens in T. congolense infected hosts (Manisfield and Wallace (1974), Holmes et al. (1974)). It has been suggested that the response to the trypanosome by the infected host may also be defective and could account in part for the parasites survival (Murray and Urquhart, 1977). Little is known about the repopulation and recovery of the lymphoid system of the infected bovine after treatment, but reinfection soon after therapy may find the animal in a poor condition to respond immunologically. In our experiments, animals were given relatively long periods to recover after treatment and under these circumstances were demonstrably resistant to challenge for relatively long periods.

It also appears that the antigenic composition of populations of T. congolense in nature is complex and the number of different strains or serodemes being transmitted in given areas at different times may play an important role in the development of immunity (Dar, et al. 1973; Wilson, et al. 1973). We detected no cross strain immunity either against blood or tsetse fly induced infections with a strain of T. congolense from a different area. Under these circumstances any cross species immunity would be extremely unlikely and the presence of different species and strains trypanosomes in the same host would probably complicate the acquisition of immunity.

The consistent induction of immunity to both blood and tsetse fly challenge over a relatively long period lends support for the postulate that alternate genes are responsible for the process of antigenic variation. Our studies indicate that there probably is a limitation imposed by the parasite genome on the occurrence of different antigenic types. This would not be consistent with a process depending on the selection of mutants (Seed, 1974). Grey (1965) showed that a relatively predictable series of predominant antigenic types of T. brucei appeared early in the course of infection in different hosts. He also described a reversion to a basic strain antigen which took place upon cyclical or

syringe passaged transmission. It appeared, however, that some tsetse flies transmitted trypanosomes with a mixture of both basic strain and variant antigen types. Other investigators have also provided evidence that metacyclic trypanosome populations are antigenically heterogeneous (Leray, et al. 1978).

Our experiments tend to support these findings since our experimental animals which were immunized by either infection and self cure or infection and chemotherapeutic treatment showed a marked resistance to tsetse fly challenge with the same strain of trypanosome. The relatively brief periods of parasitemia which occurred in some immunized animals possibly were due to the partial waning of immunity against particular predominant variant antigens or because tsetse flies transmitted populations of parasites, a portion which possessed a variant antigen not previously experienced by some of the immunized animals. Eventhough infections were established in some immunized animals they were controlled and eradicated rapidly indicating that whatever the extent of metacyclic heterogeneity, their progeny for the most part, were antigenically similar to those of previous blood induced infections.

The phenomena of self cure in younger animals and in previously infected adults is interesting and poses some important questions. The progressively decreasing level of parasitemia associated with increasing periods of apatency suggests that either the host response is increasingly efficient or the parasites capability to produce different antigenic variants eventually becomes exhausted. Whether or not similar cross reacting groups which have been identified in surface antigens from different variants (Barbet and McGuire, 1978) play a role in this increasingly enhanced ability to control the level and frequency of parasitemia in both self cured and previously infected animals is yet to be determined. In our experience, however, it would be unlikely that this immunity would extend to trypanosomes another strain.

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# RESULTS OF PRIMARY CHALLENGE OF PREVIOUSLY INFECTED AND TREATED CATTLE

1. F - Female; MC - Male Castrated.
2. Prepatent Period.
3. Time between treatment and challenge.
4. S.C. - Self Cure (Time of last patent parasitemia after challenge).  
T - Treated (Time since challenge).

TABLE 2

## RESULTS OF PRIMARY CHALLENGE OF PREVIOUSLY INFECTED, SELF-CURED ANIMALS

An. No.	Initial Infection					Interval <sup>3</sup> (Wks-Days)	Primary <sup>4</sup> Challenge (1x10 <sup>4</sup> /500Lbs.)		
	Age (Yrs)	Sex <sup>1</sup>	Dose per 500Lbs.	P.P. <sup>2</sup> (Days)	Last Patent Parasitemia (Wks-Days)		Age (Yrs)	P.P. <sup>4</sup> (Days)	Result <sup>5</sup> (Wks-Days)
1	0.5	M	2.8x10 <sup>3</sup>	8	54-4	25-0	2.0	N.P.	N.D.I.
2	1.3	MC	1.0x10 <sup>4</sup>	5	61-1	31-6	3.0	N.P.	N.D.I.
3	0.3	F	1.0x10 <sup>4</sup>	5	56-2	36-5	2.0	N.P.	N.D.I.
4	0.5	M	2.9x10 <sup>3</sup>	6	30-5	48-5	2.0	N.P.	N.D.I.
5	1.4	MC	1.0x10 <sup>4</sup>	5	38-2	54-5	3.1	N.P.	N.D.I.
Average of 3 control animals for primary challenge							3.1	4.7	T (9-3)

1. F - Female; MC - Male Castrated; M - Male.

2. P.P. - Prepatent Period.

3. Time between last patent parasitemia and challenge.

4. P.P. - Prepatent Period; N.P. - Not Patent.

5. N.D.I. - No Detectable Infection; T - Treated (time since challenge).



TABLE 1

THE EFFECT OF AGE ON TRYPANOSOMA CONGOLENSIS INFECTIONS IN CATTLE

Age (Years)	Number of Animals	Median Survival* Time (Weeks-Days)	Range (Weeks-Days)	No. Self Cures (%)
0.3-1	11	> 78-0	6-8 to > 78-0	10 (91)
1-2	11	24-4	5-5 to 30-6	2 (11)
2-3	11	11-5	5-5 to 78-0	0 (0)
3-4	5	6-3	6-1 to 13-6	0 (0)
4-5	2	6-8	4-2 to 9-0	0 (0)
5-6	2	8-1	8-0 to 8-3	0 (0)

\* Based on time to treatment or day of death.

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TABLE 2

TIME REQUIRED TO DETERMINE PRESENCE OR ABSENCE OF  
PARASITES BY CULTURE OF SPLENIC ASPIRATE MATERIAL

Cultures Done		Day Cultures Positive					
		1	2	3	4	5	6
Pre-RX	13	1	11	1	0	0	0
RX	68	3	37	15	11	2	0

TABLE 1

COMPARISON OF FIELD'S STAINED SMEARS OF SPLENIC ASPIRATE MATERIAL  
WITH PARALLEL CULTURE OF THE SAME MATERIAL

Culture	Smear	
	Positive	Positive
		Negative
Positive	Positive	60
		8
Negative	Negative	1
		0

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visceral leishmaniasis, as evidenced in this study by detection of parasites by culture in patients late in the course of therapy when the stained smears were negative.

We did not compare the two culture techniques in our studies because the standard diagnostic procedure utilized in Kenya is the examination of stained smears and not the use of NNN. Furthermore, it would have been difficult to aliquot the splenic aspirate material to properly and simultaneously evaluate the three techniques.

We believe that this culture technique offers an advantage over stained smears even in confirming a presumptive diagnosis primarily because of the ease of seeing large numbers of motile promastigotes in contrast to what can be a difficult and time consuming search for amastigotes in stained splenic aspirate smears. The greatest value of the culture lies in its use later in the course of therapy when it is quite sensitive in detecting small numbers of parasites and thus provides an objective criterion on which to judge the effectiveness of therapy.

pretreatment cultures. When cultures were taken during the course of therapy, most cultures were positive on day 3 or 4 with none becoming positive later than day 5.

#### DISCUSSION

For many years most clinical laboratories have used blood based diphasic media such as NNN for the in vitro cultivation of leishmania. From the clinician's viewpoint, these media suffer from the disadvantage of often requiring at least two weeks before leishmania can be detected in the culture. In contrast, cultures rapidly become positive in Schneider's insect culture medium. Though the blood based media have been widely used to cultivate promastigotes for biochemical and immunologic studies, these media have serious deficiencies which limit their laboratory application as well. These include cultivation of parasites with red blood cell derived antigens, inconsistent growth of different species and strains of leishmania, and often low yields of organisms.

Schneider's medium with 30% FBS has demonstrated its ability to support a wide array of *Leishmania* species ~~as well as~~ geographic strains (Hendricks et al., 1978). Recently this insect medium plus 30% (v/v) FBS has been compared to NNN in terms of its sensitivity as a culture medium. The increased sensitivity is presumably due to its ability to support in vitro transformation and multiplication of low numbers of parasites. This has been demonstrated when this culture technique was used to diagnose and evaluate patients with cutaneous leishmaniasis and the procedure was shown to be more effective than either NNN or histopathologic examination (Hendricks and Wright, 1979). Similarly, this method is more sensitive than morphologic examination in patients with



the medium was modified by the addition of 30% (v/v) heat inactivated (56°C for 30 min) fetal bovine serum (FBS) and 100 IU penicillin and 100 ug streptomycin (final concentration/ml). Parasites were cultured in 16 x 10 mm plastic tissue culture tubes containing 3.0 ml of freshly prepared medium or vaccine vials containing freeze-dried Schneider's medium and FBS were reconstituted with 3 ml distilled water and used for diagnosis as described above.

The inoculated cultures were incubated at 26°C and examined daily for the presence of promastigotes by ordinary light microscopy. Promastigotes correspond morphologically to the sandfly stages of the leishmania life cycle and are extra-cellular, flagellated, aerobic and grow optimally at temperatures below 30°C. The culture tubes are ideally observed with an inverted phase contrast microscope but ordinary light microscope with a 10x objective is adequate.

The cultures were examined for 28 days before being considered negative.

### RESULTS

Among 13 patients examined prior to treatment, smears of splenic aspirate material and parallel cultures of the same material were all positive. During the course of treatment, differences were noted between the two methods (Table 1). These techniques were compared 68 times during therapy, and in eight instances no parasites were detected by smear although the cultures were positive. A positive smear with a negative culture occurred only once.

The time required to detect parasites by culture of splenic aspirate material is shown in Table 2. Parasites were found within 72 hours in

The incidence of visceral leishmaniasis appears to be on the increase in Kenya and transmission is occurring in areas remote from sophisticated medical support. Accordingly, it seemed worthwhile to explore the use of Schneider's medium in the diagnosis and management of visceral leishmaniasis. This study was itself part of a broader study of the clinical aspects of leishmaniasis including diagnosis and evaluation of standard antimony therapy (Kager et al., In Preparation; Rees et al., In Preparation).

### PATIENTS, MATERIALS AND METHODS

#### Patients

All patients seen by us over a period of 6 months at the Kenyatta National Hospital having visceral leishmaniasis were included in this study.

#### Splenic Aspirates

Splenic aspirates were performed using a standard technique (Kager, et al., In Preparation). An aspirate was performed prior to beginning treatment and at weekly intervals during the course of therapy. A small quantity of aspirate material, often amounting to little more than minute drops, was expressed into the culture medium from the aspirating syringe and needle. Careful aseptic technique was used to keep the aspirate and medium free from contamination. A further amount of splenic aspirate material was then expressed onto a glass microscope slide. A thick smear was made and stained with Field's stain and subsequently examined by light microscopy.

#### Culture Procedures

Commercially prepared Schneider's insect cell culture medium was used in all experiments (Schneider, 1974). Immediately prior to use

The Culture of *Leishmania donovani* in Schneider's Insect Medium:  
Its Value in the Diagnosis and Management of Patients with  
Visceral leishmaniasis

INTRODUCTION

Diagnosis of visceral leishmaniasis has usually involved demonstration of the parasite in stained smears of various tissues or body fluids (Manson-Bahr, 1972). In most instances, the material is obtained by bone marrow or splenic aspirates. Alternatively, serologic techniques such as indirect hemagglutination, indirect fluorescent antibody, complement fixation and micro-Elisa are considered to be adequate (Kagan and Norman, 1976; Hommel et al., 1978) for routine diagnosis but are rarely employed.

The first report of the culture of Leishmania donovani was made in 1904 using citrated blood (Rogers, 1904). In 1908, Nicolle reported a simplification of the Novy and MacNeal medium (Nicolle, 1908a; Nicolle, 1908b). This simplification became known as NNN and with minor variation has been the mainstay of leishmanial culture in clinical practice for the past 70 years. NNN consists of distilled water and blood agar but without peptones and beef. Cultures may become positive by the third day on NNN but often take as long as 3 to 4 weeks. The long delay in awaiting results for both diagnosis and assessment of cure has limited the value of this culture medium for the clinician. Various hemoprotozoa culture methods employing a variety of different media have been introduced during the past few years (Dwyer, 1972; Cross and Manning, 1973; Mansour et al., 1973; Miller et al., 1974; O'Daly, 1975; Steiger and Steiger, 1976; Hendricks et al., 1978) but for practical reasons or because of their cost, they have not yet gained widespread acceptance. The use of Schneider's insect culture medium, however, has been shown to be simple to use and valuable in the diagnosis of cutaneous leishmaniasis (Hendricks and Wright, 1979).

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TABLE 4

RESULTS OF THE SECOND CHALLENGE OF BOVINES REQUIRING TREATMENT  
OR SELF-CURED AFTER PRIMARY CHALLENGE

Group	Animals (No.)	Interval <sup>1</sup> (Months)	Prepatent <sup>2</sup> Period (Days)	Result <sup>3</sup> (Weeks-Days)
Self cure	3	5-30	N.P.	N.D.I.
Treated	3	6-10	N.P.	N.D.I.
Control (Average)	6	-	5.5	T (8.0)

1. From last patent parasitemia or treatment.

2. N.P. - Not Patent.

3. N.D.I. - No Detectable Infection; T. - Treatment (time after challenge).

TABLE 5

RESULTS OF TSETSE FLY CHALLENGE OF ANIMALS IMMUNIZED  
AGAINST BLOOD FORMS OF TRYPANOSOMA CONGOLENSE

Group	Age (Range) Years	No. Patent/ No. Challenged	Prepatent Period (Range)	Days of <sup>2</sup> Patent Infection (Range)	Lowest <sup>3</sup> PCV (%) (Range)	Result <sup>4</sup>		
						N.D.I.	S.C.	T.
Immune	4.6(1.9-8.0)	7/12	20.1(6-56)	6.4(0-32)	29.1(25-34)	5	7	0
Partially Immune <sup>1</sup>	5.4(4.2-6.3)	3/3	15.3(13-19)	79.7(61-100)	20.3(18.5-22.5)	0	2	1
Controls	3.5(1.2-7.4)	9/9	10.8(10-13)	96.6(83-100)	17.3(15.5-19.5)	0	1	8

1. Animals having a single infection and treatment.

2. First 100 days after day 10.

3. Packed cell volume.

4. N.D.I. - No Detectable Infection; S.C. - Self Cure; T. - Required Treatment.

TABLE 6

## HISTORY OF ANIMAL 151

Procedure	Date	Infection		Prepatent <sup>3</sup> Period (Days) 151 (control)	Result <sup>4</sup> (Days)	Lowest <sup>5</sup> P.C.V. (%)
		Source <sup>1</sup>	Method <sup>2</sup> Dose/ 500lbs.			
Primary Infection	14-9-70	B	S	5 (5)	T. (196)	11.0
1st Challenge	11-8-72	M	S	14 (5)	S.C. (32)	31.5
2nd Challenge	5-3-75	M	S	N.P. (5)	N.D.I.	31.0
3rd Challenge	10-7-75	B (Relapse)	S	5 (3)	S.C. (34)	27.0
4th Challenge	17-3-76	M	S	N.P. (5)	N.D.I.	30.0
5th Challenge	15-12-76	M	S	N.P. (5)	N.D.I.	29.0
6th Challenge	26-9-77	B	T	(19 (11)	S.C. (21)	33.0

<sup>1</sup>B - Bovine; M - Mouse. <sup>2</sup>S - Syringe; T - Tsetse fly. <sup>3</sup>N.P. - Not Patent.

<sup>4</sup>T - Treated (Days since challenge); S.C. - Self Cure (last patent parasitemia);  
N.D.I. - No Detectable Infection.

<sup>5</sup>P.C.V. - Packed Cell Volume.

TABLE 7

RESULTS OF BLOOD AND TSETSE FLY INDUCED CHALLENGE OF ANIMALS IMMUNE  
TO THE TRANS-MARA I STRAIN OF TRYPANOSOMA CONGOLENSIS  
WITH A HETEROLOGOUS STRAIN (YOANI-I)

Group	Type Challenge	No. Patent/ No. Challenged	Prepatent Period (Range)	Result <sup>1</sup>		
				N.D.I.	S.C.	T.
Immune	Blood	3/3	7.3(6-9)	0	0	3
Control	Blood	3/3	7.0(6-8)	0	0	3
Immune	Tsetse Fly	3/3	13.6(12-15)	0	0	3
Control	Tsetse Fly	3/3	13.6(13-14)	0	0	3

1. N.D.I. - No Detectable Infection; S.C. - Self Cure;  
T. - Required Treatment.



Fig.1

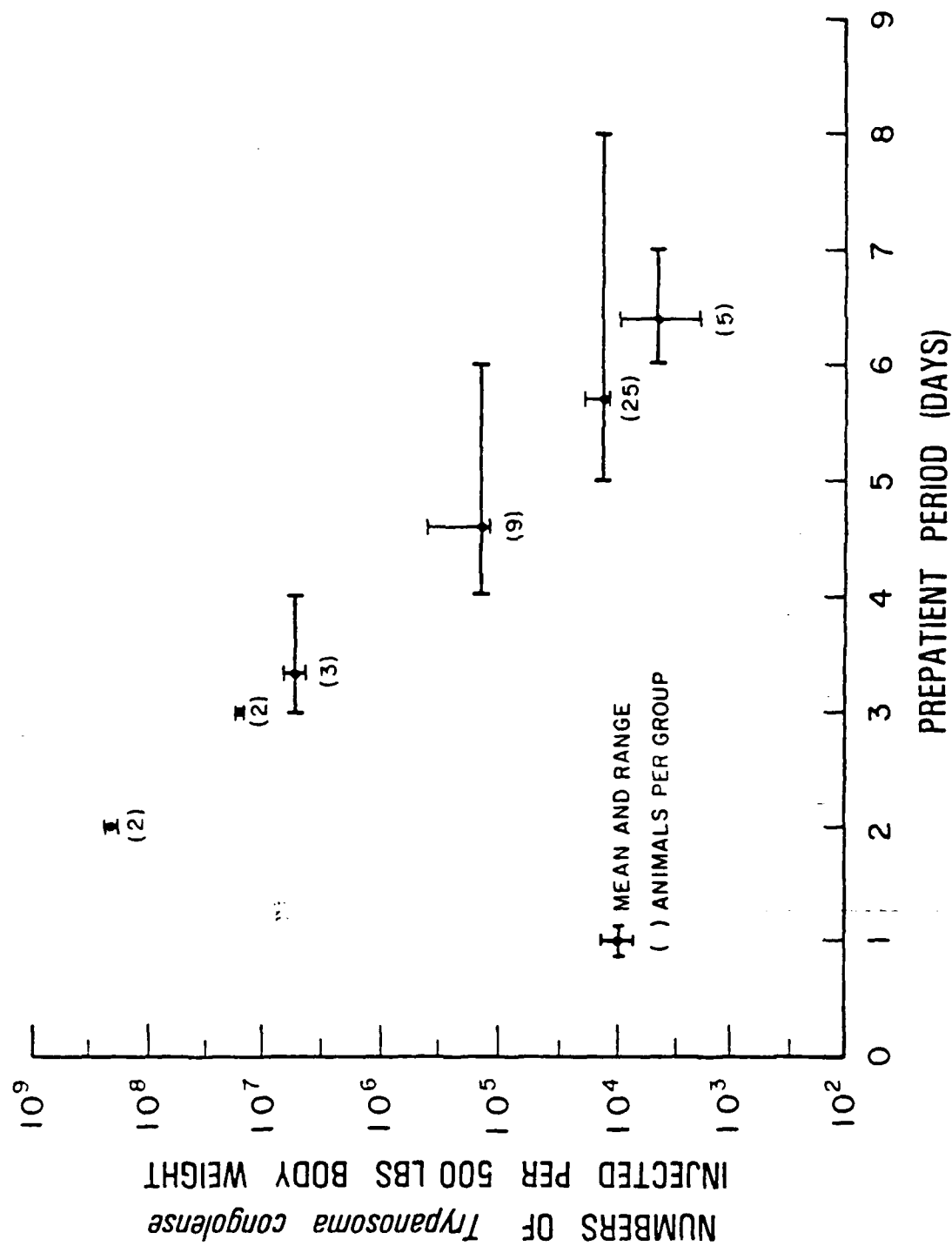
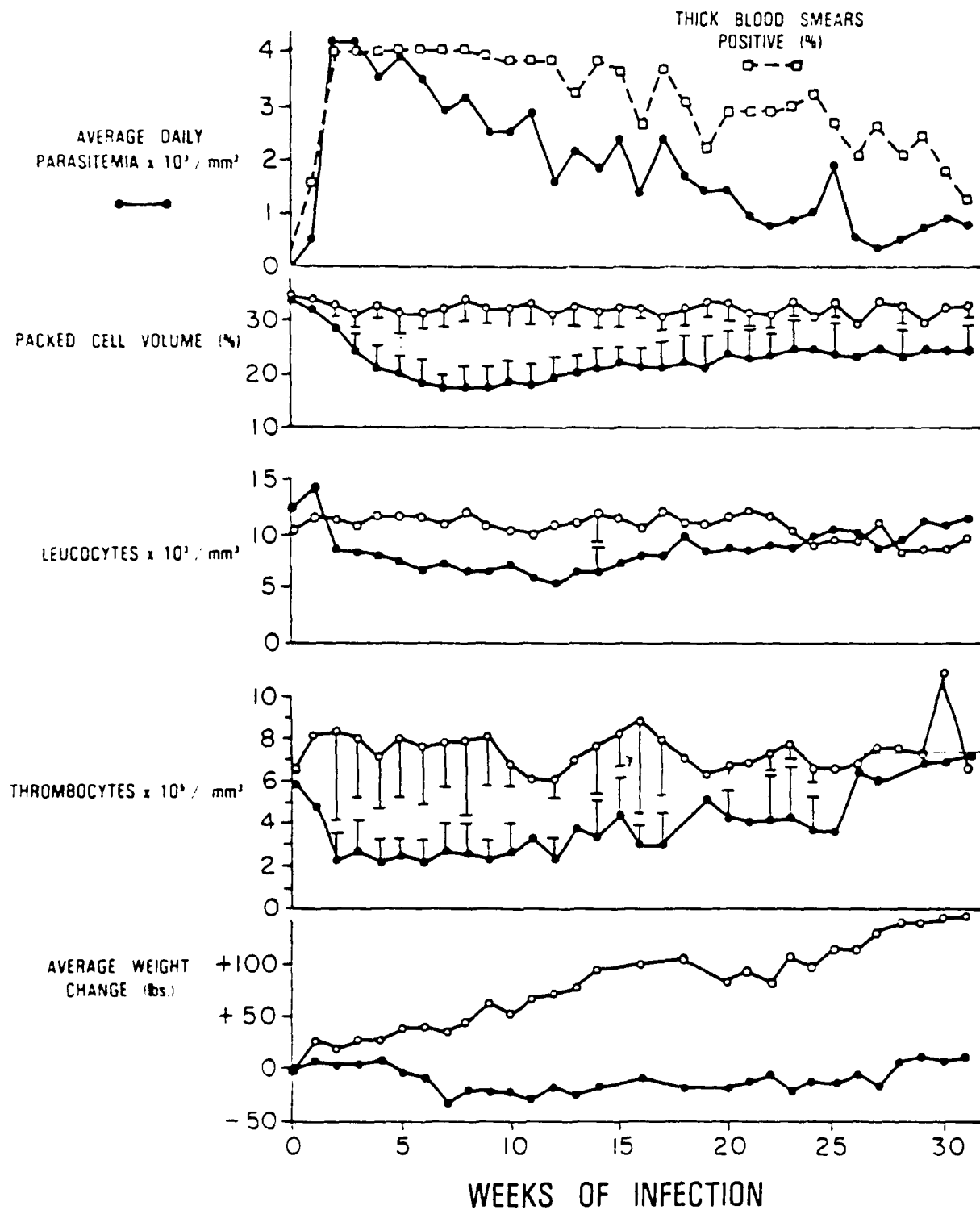


Fig. 2



WEEKS OF INFECTION

Fig. 3

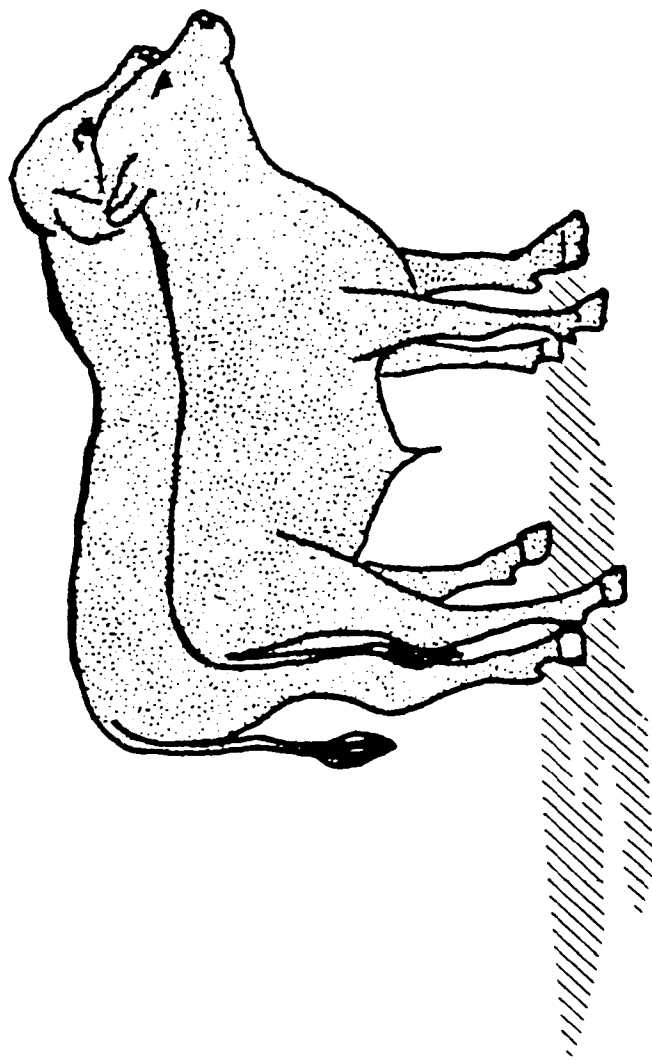


Fig. 4

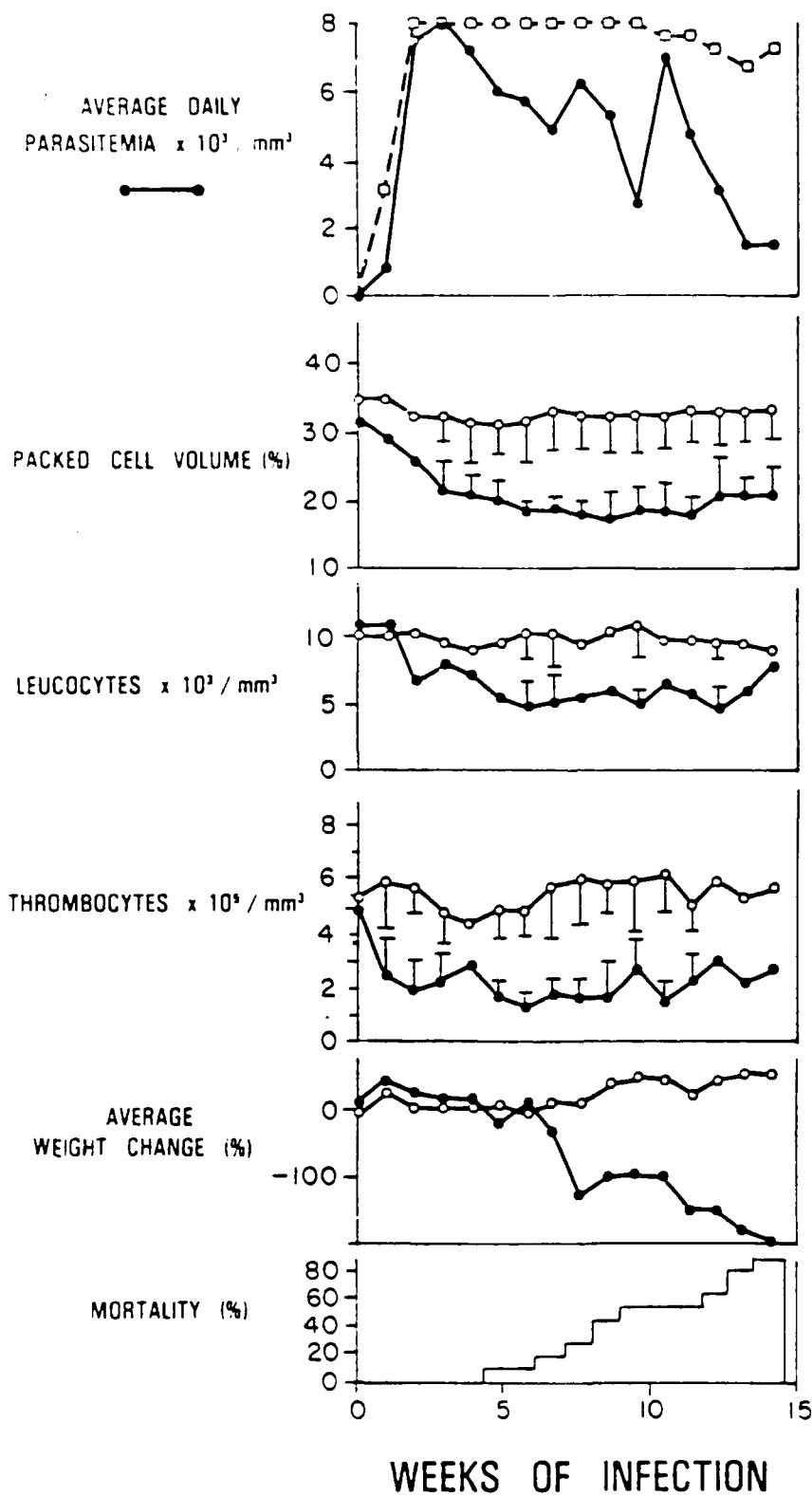


Fig. 5

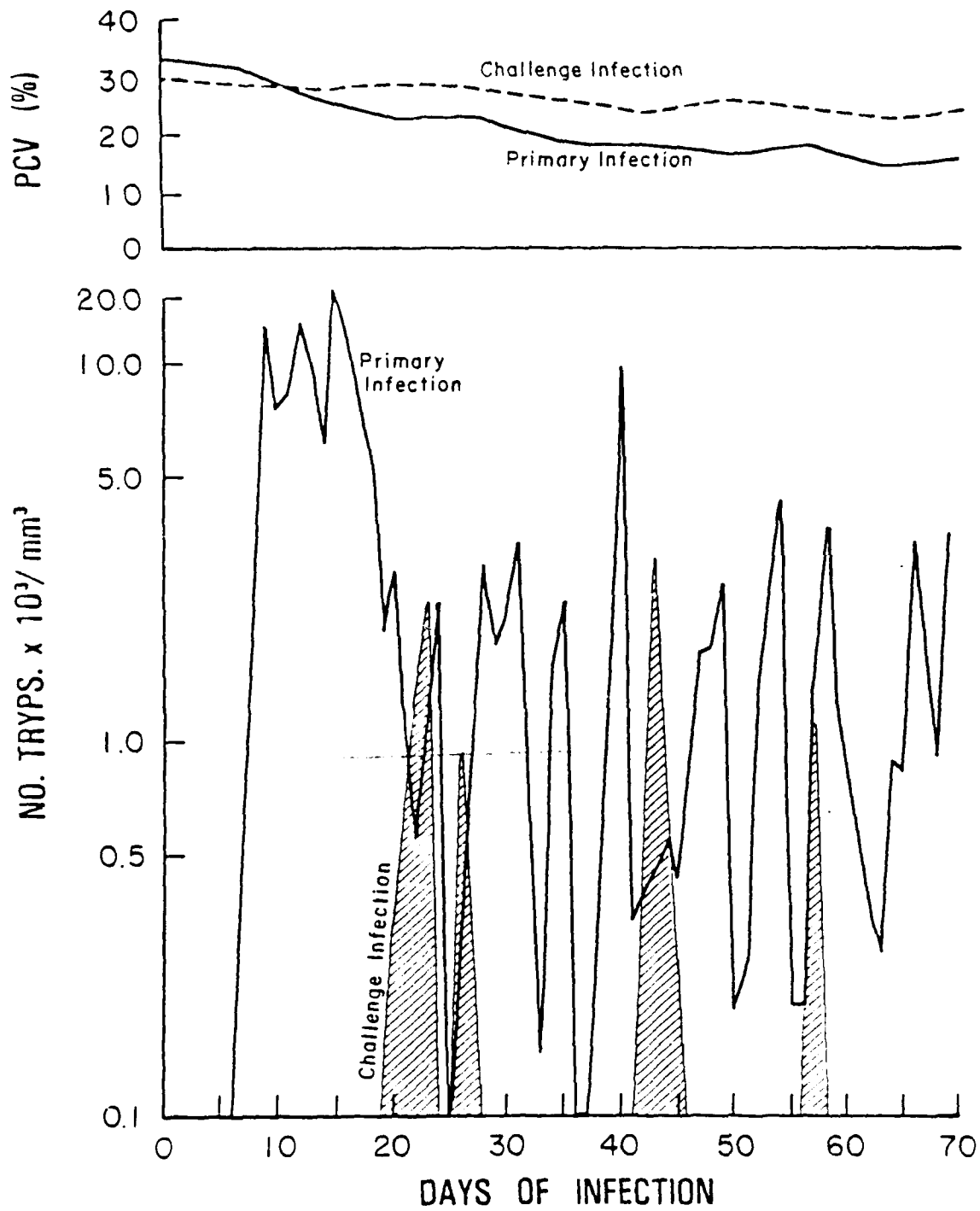


Fig.6

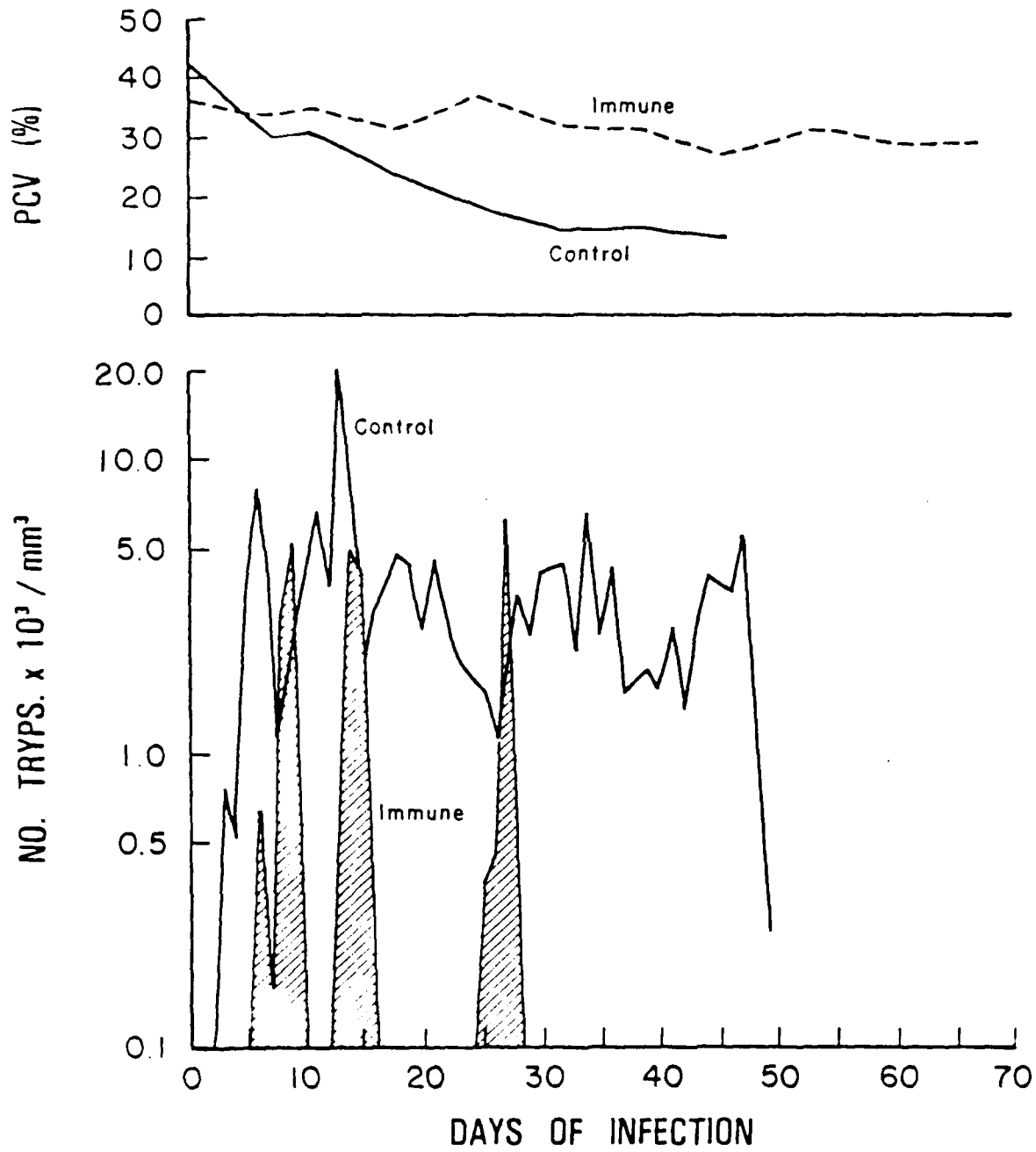


Fig. 7

PATTERNS OF PARASITEMIA IN 5 IMMUNIZED BOVINES  
AFTER TSETSE CHALLENGE

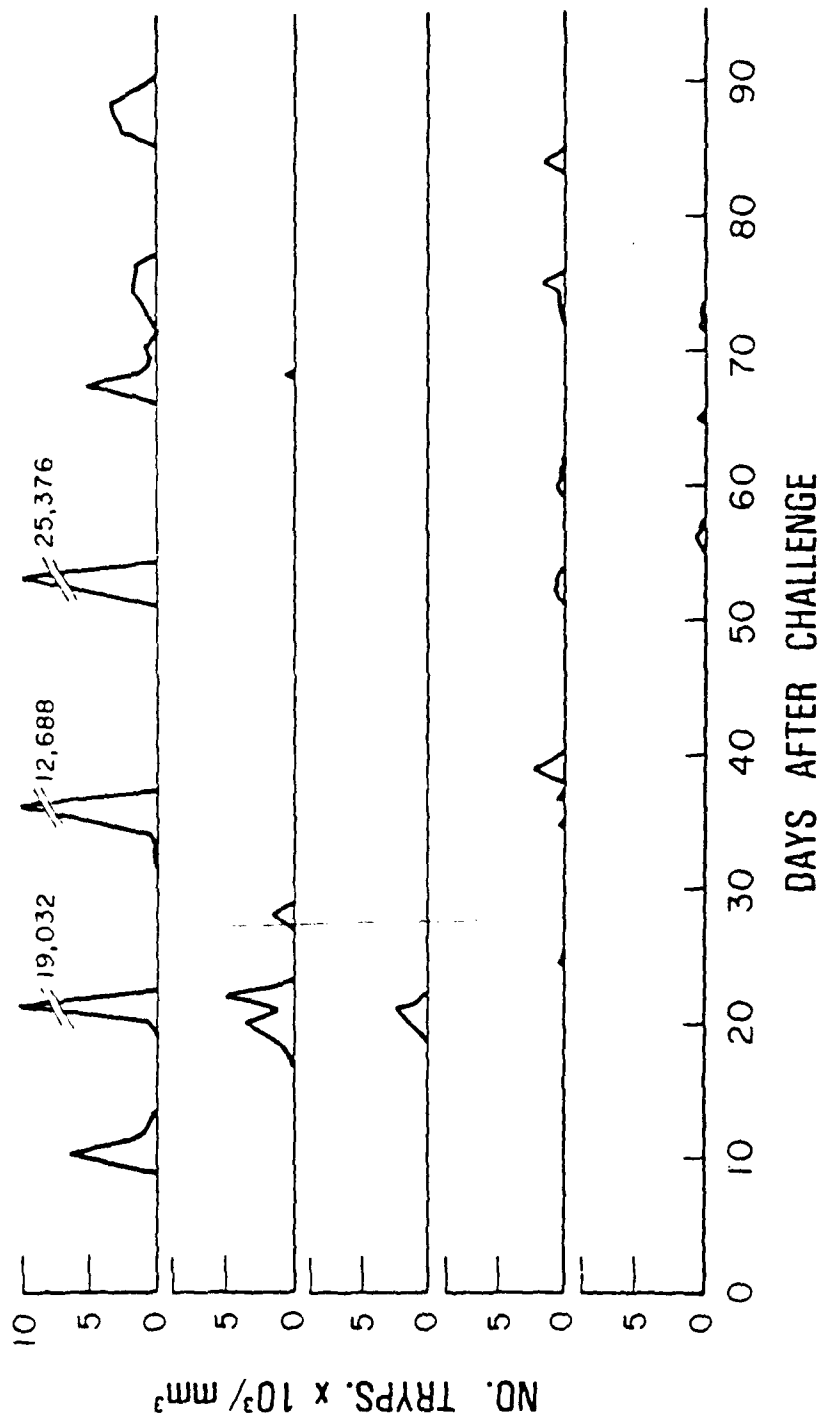


Fig. 8

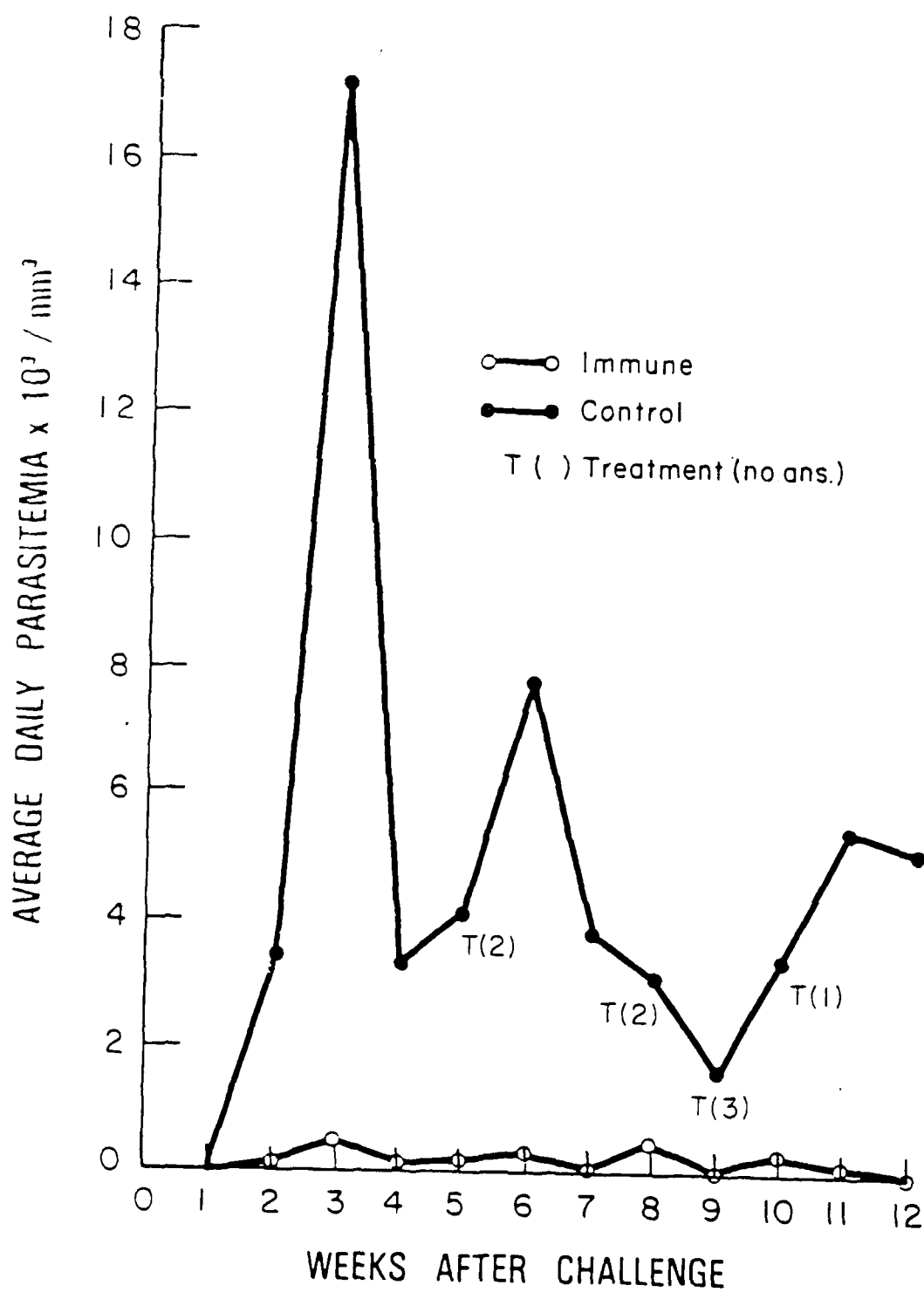




Fig. 9

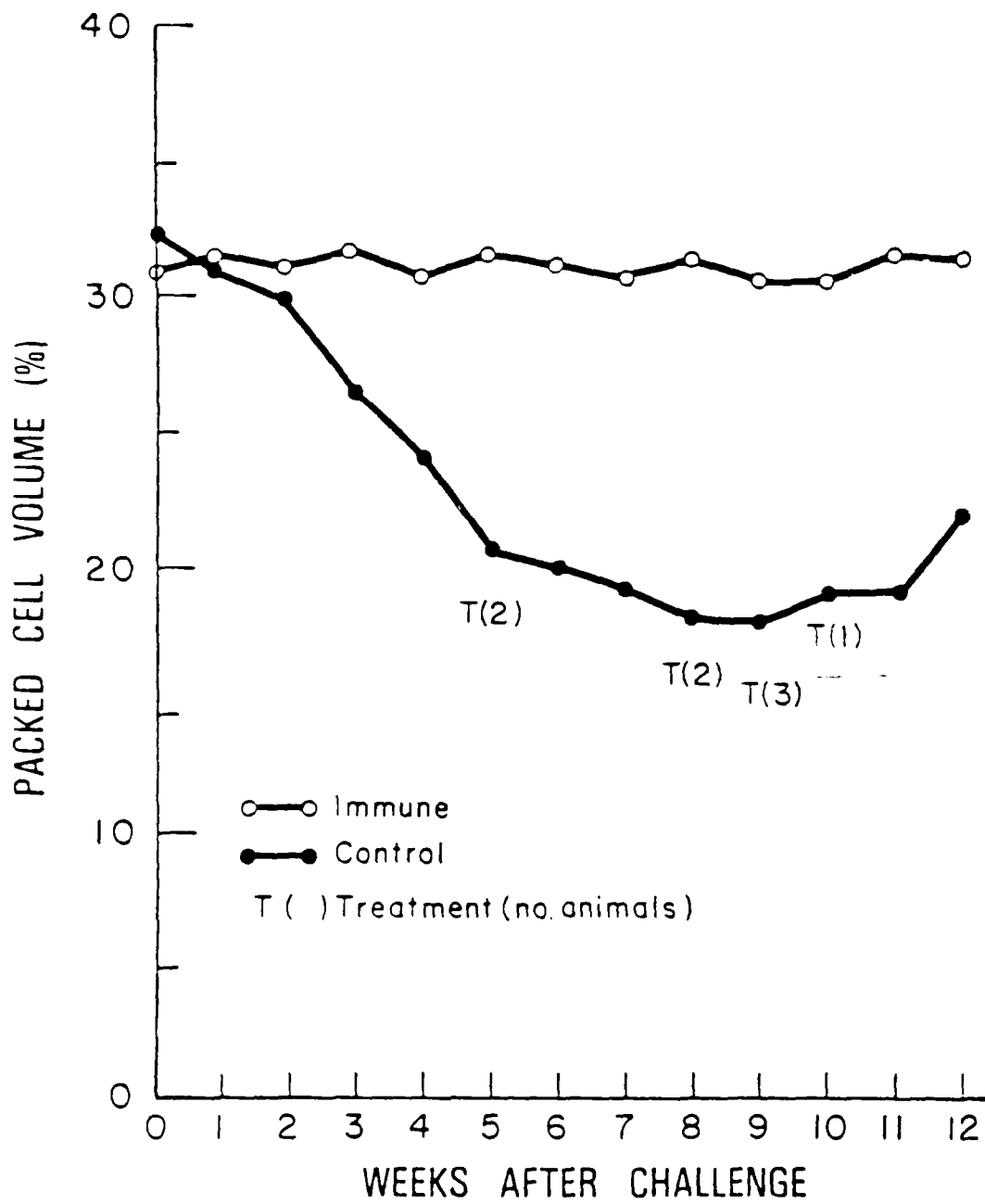
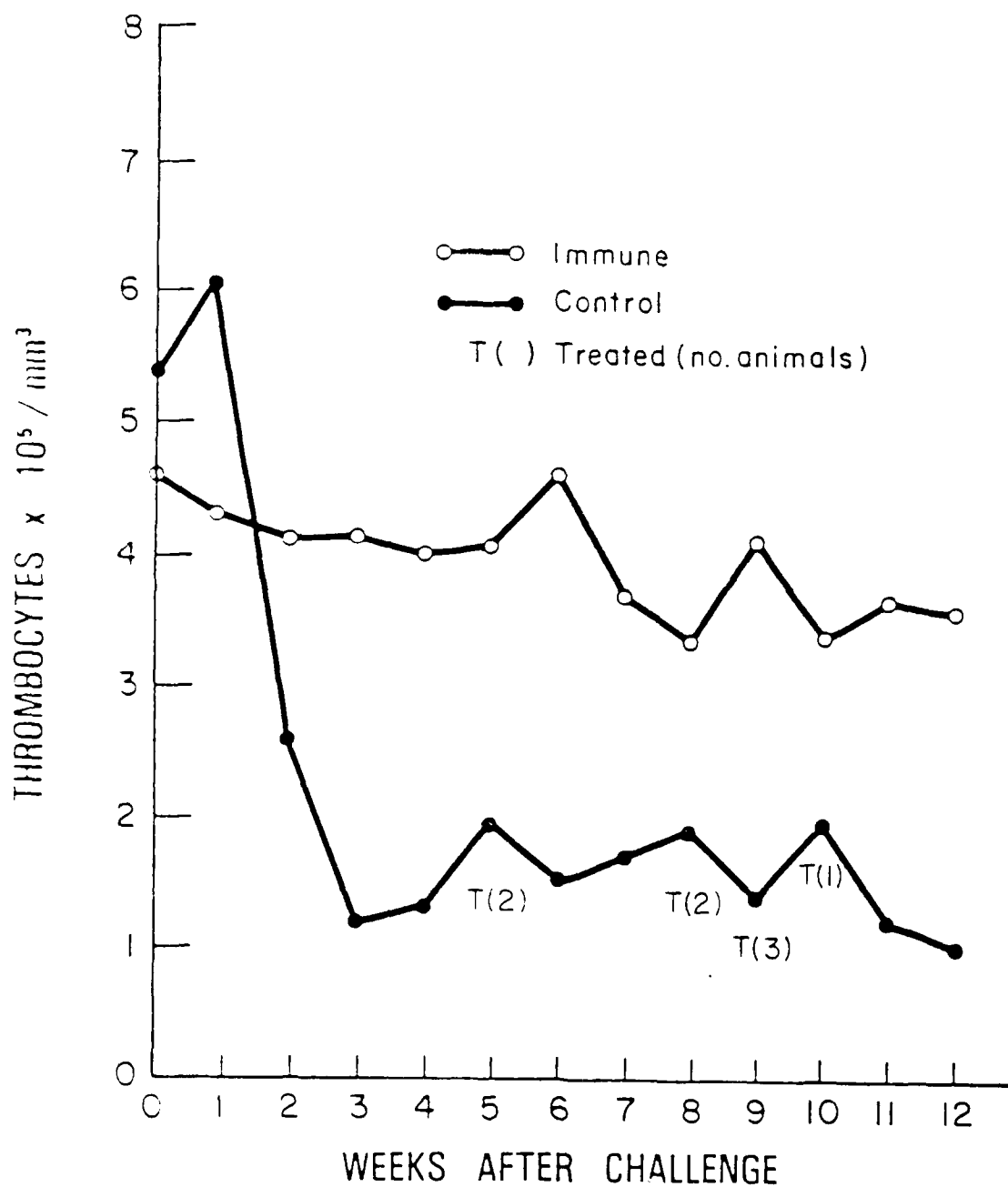


Fig. 10



### T. congolense Infection

The dissection results for randomly selected cages of flies, 25-45 days old, is shown in Table 1. Male flies acquired cyclic infections more frequently than female flies. Midgut infections occurred more frequently than proboscis infections, and we did not find any proboscis infection without a midgut infection. Numbers of trypanosomes in the proboscis varied from  $<50$  to  $>1,000$ .

### Fly Infection Experiments

Feedings on a T. congolense infected host beyond the initial teneral feeding produced a greater number of cyclic infections in G. morsitans (Table 2). The rate of increase in female flies (17.2% with one exposure vrs. 30.4% with 4 exposures) was more pronounced than that for males (26% with one exposure vrs. 31.6% with 4 exposures).<sup>6</sup> Host parasitemia during the 5 exposures was calculated to be  $2 \times 10^6$  organisms per cc of blood.

### Fly Transmission to Rats

Of the 60 flies fed individually on rats, 12 (20%) transmitted a detectable infection. Dissections demonstrated that 2 of the 48 flies which failed to transmit had trypanosome infections in the proboscis and midgut. Table 3 shows the results of individual refeedings of the 12 infected flies on laboratory rats. Five of the 12 transmitted T. congolense at each feeding, while 2 flies which transmitted in the initial feeding failed to transmit in any subsequent feedings. The prepatent period after fly feeding varied from 10-27 days. Of the rats developing a parasitemia after fly feeding 71 of 75 (94.7%) died from the infection. Those rats not showing a parasitemia were challenged with T. congolense blood forms harvested from mice. None survived challenge.

### DISCUSSION

Potts (1933) reported an 87% emergence rate for G. morsitans puparia deposited in the laboratory. Buxton and Lewis (1934) compared the weight of one day old puparia to emergence of adult Glossina tachinoides and found the highest emergence (84.2%) from the heaviest group of puparia. It appears from rearing results with our colony that intensive feedings on a live bovine host is an ideal system in terms of G. morsitans production. Pregnant female flies just prior to larviposition should be avoided for studies requiring feeding on a host animal. These flies probably account in part for the decline in the feeding rate (100 - 92%) observed in the 54 sequential exposure of flies to a host.

In both the dissections of randomly selected cages of flies and the refeeding of flies on an infected host we found higher cyclic infection rates in males than in females. Studies by Burt (1946) with G. morsitans and T. rhodesiense indicated that in males, cyclical infection was 2.5 times as frequent as in females. In the present study, the difference

## Infection Experiments

Five groups of approximately 40 newly emerged (teneral) flies each were fed on a T. congolense-infected calf. On each subsequent day, one group was transferred to a negative animal while the remaining groups were refed on the infected animal. This was continued until day 6 when all cages were fed on a negative animal. Thus, group I fed on an infected animal once, group II fed twice on an infected animal, group III - 3 times, group IV - 4 times and group V - 5 times. Wet mounts prepared from blood of infected and negative animal were checked daily before flies were fed. Parasitemia of the infected animal was estimated daily by counting the numbers of trypanosomes per 100 leucocytes on thick blood films and relating these numbers to the total leucocyte counts per mm<sup>3</sup>.

## Fly Transmission to Rats

Three cages of 20 G. morsitans each were fed from emergence ( 24 hours old) until day 17 on a T. congolense infected calf. The cages were then fed on a non-infected bovine for 7 days to insure that subsequent transmission by individual flies was the result of a cyclic infection with T. congolense. Each fly was transferred to an individual plastic tube with a cork stopper at one end and screen mesh at the other. The flies were then fed singly on white rats to determine which flies could transmit a detectable infection to the rats. Infected flies that initially transmitted to rats were fed on a fresh rat each day for 10 consecutive feedings or as many as possible before the flies died. Wet blood smears from the host rats were examined daily from day 10 after fly feeding. Rats without parasitemia were challenged at day 30 with a normally lethal dosage of blood forms of T. congolense. After 10 consecutive feedings, the infected flies remaining alive were pooled for mating, and the progeny were maintained as a separate group from the main colony.

## RESULTS

### Fly Colony

No discernable differences were observed between flies reared from Bristol puparia and those from the Walter Reed colony. The colony produced an average of 3,616 flies per month (range: 3,014 to 4,426) during FY 79. Pupal weight for randomly sampled one day old pupae was 27.9 mg. (N = 2,830). Adult flies successfully emerged from 2,546 (90%) of these pupae. Flies invariably fed on the host animal at each feeding interval for the first week post emergence, but in 54 sequential host exposures of 10 cages of flies the overall feeding rate was 90.2%. Pregnant females just prior to larviposition often failed to feed during the 15-minute exposure to a host. We occasionally observed flies which had fed to repletion on the host, but appeared unable to digest the blood meal. Such flies ceased to feed during subsequent exposures on a host and died 2-3 days later. A few flies were observed to engorge to such an extent that they died within minutes after feeding.

TRYPANOSOMA CONGOLENSE: FACTORS AFFECTING INFECTION AND  
TRANSMISSION IN LABORATORY-REARED GLOSSINA MORBITANS

INTRODUCTION

Factors known to influence tsetse fly infection rates include the species of fly and trypanosome, fly age at exposure to trypanosomes, temperature and the type of host animal. Buxton (1955) and Jordan (1976) suggested that there also may be certain genetic lines of tsetse flies within a species that become infected more readily than others. For a cyclically infected fly to transmit trypanosomes, sufficient quantities of infective metacyclic forms must be passed to a susceptible host during fly feeding. This report describes studies of some factors influencing both cyclic infection of Glossina morsitans Westw. with Trypanosoma congolense and subsequent transmission from fly to vertebrate host. Specific objectives were to determine (1) if the number of exposures to an infected host influences T. congolense infection in the fly; (2) if the sex of the fly influences infection, and (3) the rate of transmission by individual infected flies during feedings to reptiles on susceptible rats. Incident to these studies, a selective fly rearing program was initiated in an attempt to establish a genetic line more susceptible to cyclic infection with T. congolense.

MATERIALS AND METHODS

Fly Colony

The Glossina morsitans colony was established from puparia supplied by Dr. A.M. Jordan, Tsetse Research Laboratory, University of Bristol, Bristol, England. The Walter Reed colony became self-sustaining during FY 79, and shipments from Bristol were discontinued. Standard rearing procedures were used except that flies were not sexed, and males and females were held in the same cages for the entire adult life. Adults were kept in incubators at  $26 \pm 1^\circ\text{C}$  and  $60 \pm 10\%$  RH. Each Geigy-type cage of 20 flies was placed on a live bovine host for 15 minutes per day, 6 days per week.

Trypanosomes

The fly colony was infected with the Trans Mara I strain of Trypanosoma congolense. This strain was isolated from an infected cow in the Trans Mara area near the Kenya-Tanzania border in 1966 (Wellde, et al. 1974). The strain has never been cloned, and the infection has been maintained in an intensive fly-bovine - fly cycle since 1979. Randomly selected cages of 20 flies, 25-45 days old, were dissected periodically to determine infection rates in the colony.

Fig. 2

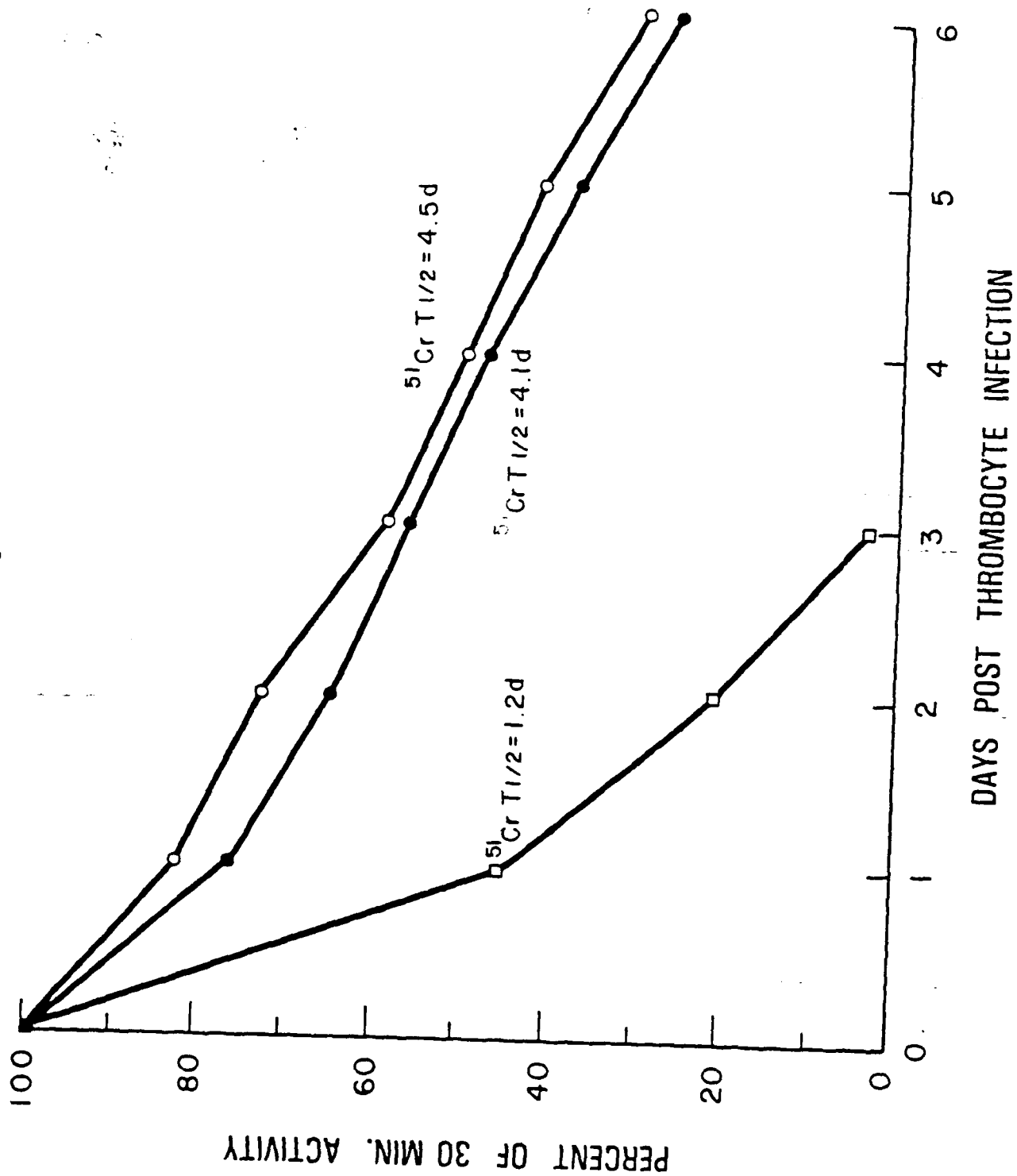
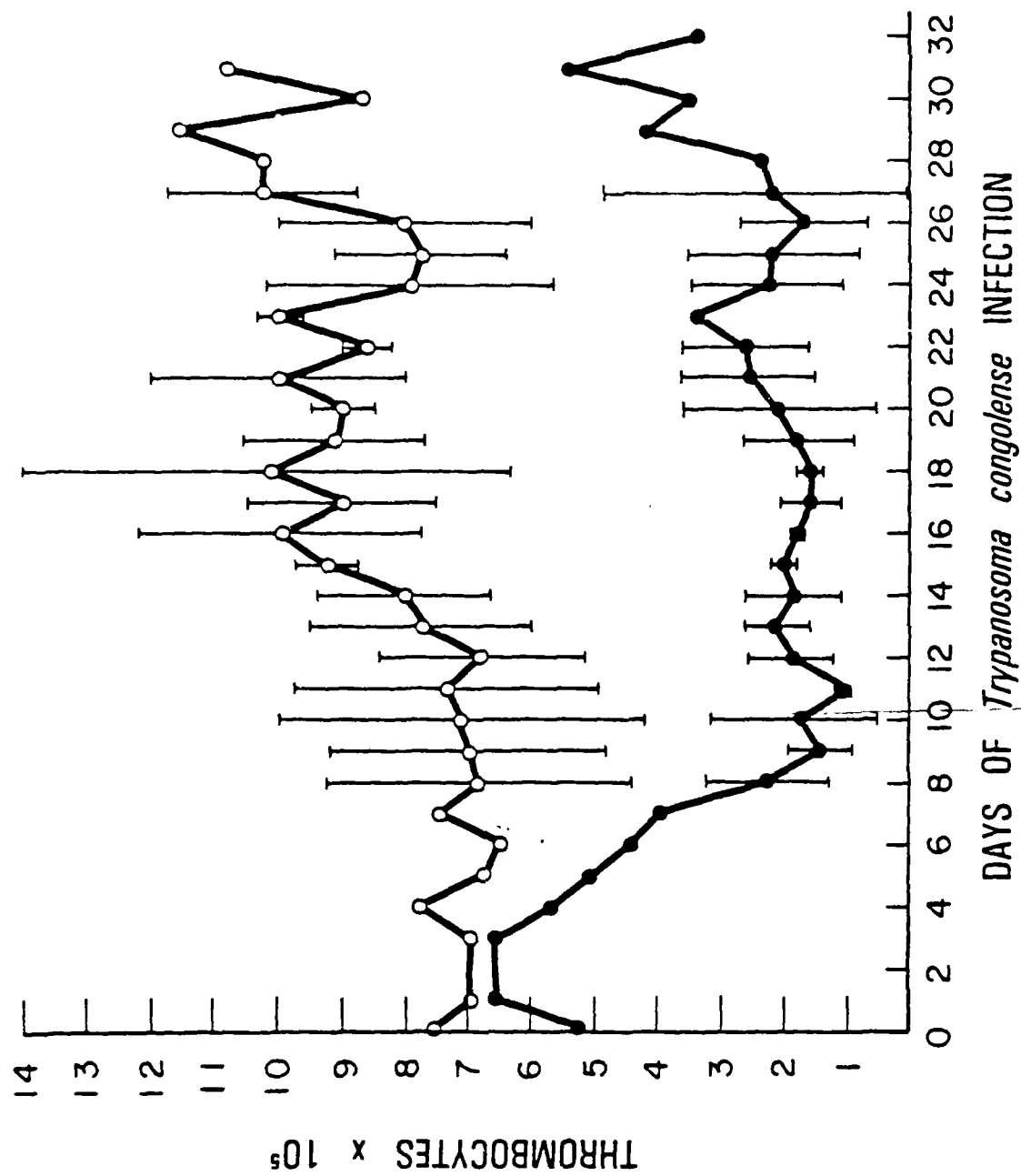


Fig. 1



LEGENDS FOR FIGURES

FIGURE 1: Thrombocyte levels in five Charolais steers infected with Trypanosoma congolense and five non-infected control steers ( $\pm 2SE$ ).

FIGURE 2: Apparent thrombocyte survival times ( $^{51}\text{CrT}_4$ ) of labelled thrombocytes in three steers:

- (a) an autologous transfusion in a normal steer (O)
- (b) a heterologous transfusion in a normal steer (O) and
- (c) a heterologous transfusion in a Trypanosoma congolense infected steer ( ).



TABLE 1

APPARENT THROMBOCYTE SURVIVAL TIMES OF STEERS INFECTED WITH  
TRYPANOSOMA CONGOLENSIS AND NON-INFECTED CONTROLS

Group	Animal No.	Prepatent Period (Days)	Day of Cr <sup>51</sup> Thrombocyte Transfusion <sup>a</sup>	Recipients Thrombocyte level at Transfusion (x10 <sup>3</sup> )	Apparent Thrombocyte Survival (T <sub>1/2</sub> ) (Days)
Infected	1	5	10	100	0.8
	2	5	14	148	1.6
	3	6	15	200	1.2
	4	5	21	182	0.9
	5	6	25	348	2.1
	Mean $\pm$ 2SE	5.4	-	195.6 $\pm$ 83.5	1.3 $\pm$ 0.5
Control	6	-	-	1042	2.9
	7	-	-	1154	3.7
	8	-	-	1304	4.1
	9	-	-	580	4.5 <sup>b</sup>
	10	-	-	910	3.5
	Mean $\pm$ 2SE	-	-	998.0 $\pm$ 245.9	3.7 $\pm$ 0.5

<sup>a</sup> Days post infection.

<sup>b</sup> Autologous transfusion.

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to that of the autologous transfusion. However, the autologous transfusion produced the longest apparent survival time (4.5 days). Whether or not the somewhat shortened survival times resulting from heterologous transfusions were the result of incompatibility of antigens on the thrombocytes is not known. In man, A and B blood group antigens are present on thrombocytes and a shortened thrombocyte survival was noted when A thrombocytes were transfused into O recipients (Aster, 1965). Some clinicians, however, do not consider ABO incompatibility to be a major drawback in the treatment of thrombocytopenic patients (Breckner and Aster, 1972). Both thrombocyte specific antigens and HL-A antigens on thrombocytes can induce antibody formation in the recipient and sensitization may follow repeated transfusions resulting in greatly shortened thrombocyte survival times (Wintrobe, 1975). Since our experimental animals received only a single transfusion and were studied for a relatively short period, it seems unlikely that these antigens played an important role in the thrombocyte survival time.

The mechanism of thrombocyte destruction associated with thrombocytopenia in Trypanosoma congolense infected cattle remains to be defined. We have shown that there is a mild coagulopathy associated with experimental infections in bovines infected with the Trans Mara I strain of Trypanosoma congolense (Wellde, et al. 1978) which may have been initiated by thromboplastic substance which were generated by the persistent destruction of thrombocytes. It did not appear, however, that the coagulation process initiated the thrombocytopenia.

Rat thrombocytes aggregated when trypanosomes or supernatants from lysed Trypanosoma rhodesiense were added to thrombocyte suspensions (Davis, Robbins, Weller and Braude, 1974). A heat labile factor presumably of parasite origin apparently facilitated aggregation, sequestration and destruction of thrombocytes in their experimental rats.

Thrombocyte pooling in the spleen has been identified as an important factor in the thrombocytopenia of human trypanosomiasis (Robbins-Browne, Schneider and Metz, 1975). Thrombocytopenia, however, is prominent in splenectomized T. congolense infected calves (M.S. Bhogal and B.T. Wellde, unpublished data). However, this data did not indicate whether thrombocyte pooling occurred elsewhere in the reticulo-endothelial system.

Labelled erythrocytes also have a decreased apparent survival time in calves infected with T. congolense (Preston, Wellde and Kovatch, 1979) and the process of destruction may be similar to that of thrombocytes. Further efforts in our laboratory to elucidate the effects of trypanosomes and their products on erythrocyte and thrombocyte viability are in progress.

on the third day after infection the average thrombocyte level in infected animals progressively decreased to  $1 \times 10^5/\text{mm}^3$  on the eleventh day post infection. Thrombocyte levels in infected animals usually remained between  $1 \times 10^5$  and  $3 \times 10^5$  during the remainder of the experiment, while thrombocyte levels in control animals did not decrease over pre-infection values (Fig. 1).

#### Thrombocyte survival

In the first experiment thrombocytes were obtained from a normal healthy donor, labelled with  $^{51}\text{Cr}$ , and separated into three aliquots. One aliquot of labelled thrombocytes was returned to the donor animal while the other two aliquots were injected into a normal control and an infected animal respectively. The results of this experiment (Fig. 2) indicated that transfusions of labelled thrombocytes into autologous and heterologous normal animals resulted in similar thrombocyte half lives while the thrombocyte half life in the infected animal was substantially reduced. In subsequent experiments thrombocytes were harvested from normal animals, labelled and transfused into heterologous infected and control animals. The apparent half life of labelled thrombocytes in 5 infected animals ranged from 0.8 to 2.1 days. Survival times of thrombocytes in 5 non-infected animals, however, ranged from 2.9 to 4.5 days (Table 1). Both infected and control animals tolerated the thrombocyte transfusions without untoward reactions.

#### DISCUSSION

We have shown that there is a marked reduction in the apparent survival time of thrombocytes in bovines infected with Trypanosoma congolense. The apparent half-life of normal bovine platelets labelled with  $^{51}\text{Cr}$ -chromate was  $1.3 \pm 0.5$  (2SE) days in infected animals compared to  $3.7 \pm 0.5$  days in controls. This supports our previous report that the thrombocytopenia observed in bovines infected with Trypanosoma congolense appeared to result from increased production and destruction rather than from a suppression or inhibition of production (Wellde, et al. 1978). These results are similar to those of Robbins-Browne, Schneider and Metz (1975) who described a shortened apparent survival time of thrombocytes in humans infected with Trypanosoma rhodesiense. Other authors, however, have characterized the thrombocytopenia in Trypanosoma congolense infected calves as being due to an ineffective thrombopoiesis since platelet life span, as measured by  $^{35}\text{S}$ -methionine incorporation was normal, in spite of an increased megakaryocytic mass (Fosberg, Valli, Gentry and Donworth, 1979).

Thrombocyte transfusions in our experiments were done without regard to antigens present on the thrombocytes. Heterologous thrombocyte transfusions in normal animals resulted in similar survival times

#### Preparation of labelled thrombocytes

Twelve hundred ml. of blood was collected from healthy non-infected donor animals into a flask containing 200ml of acid citrate dextrose solution (Aster and Jandl, 1964). The blood was transferred to 400ml centrifuge bottles and centrifuged at 400g for 15 minutes. The supernatant plasma (thrombocyte rich) was then placed in 50ml tubes and centrifuged for 5 minutes at 500g to further remove contaminating erythrocytes and leucocytes. The resulting supernatant was centrifuged for 15 minutes at 1300g, the supernatant decanted and the thrombocytes resuspended in normal plasma. The thrombocyte suspension was incubated with  $1\mu\text{Ci}^{51}\text{Cr}$  as sodium chromate (high specific activity) for 15 min. with occasional mixing. The suspension was then centrifuged at 1300g and the supernatant removed. The labelled platelets were then washed twice with 50ml of normal plasma before resuspension in 30ml of plasma containing 60mg of ascorbic acid prior to injection. All procedures were carried out at room temperature.

#### Injection and sampling

Known numbers of  $^{51}\text{Cr}$ -labelled thrombocytes ( $2.1-3.6 \times 10^{11}$ ) in suspension were injected into experimental and control steers by means of a jugular catheter. Blood samples (10ml) were collected into EDTA from the contralateral vein 30 minutes after injection and daily for 7 days. Aliquots of blood (5ml) were diluted in 10ml 0.01N NaOH for scintillation counting.

Transfusions of labelled thrombocytes from a donor to another animal are referred to as heterologous transfusions. Thrombocytes obtained from a donor, and after labelling, returned intravenously to the same donor are described as autologous transfusions.

Thrombocyte counts were done using the method described by Brecker and Cronkite (1950).

#### Calculations and Expression of Results

The radioactivity of each blood sample was expressed as a percentage of the value at 30 minutes after injection. The apparent thrombocyte half-life ( $T_4$ ) was obtained by regression analysis of the disappearance curve over a 7-day period.

### RESULTS

#### Thrombocyte levels

Prepatent periods of infected animals ranged from 5 to 6 days and thrombocyte levels in these animals began to decline shortly before trypanosomes were detected in the blood. From a level of  $6 \times 10^5/\text{mm}^3$

TRYPANOSOMA CONGOLENSE: THROMBOCYTE SURVIVAL  
IN INFECTED STEERS

INTRODUCTION

Thrombocytopenia is a characteristic of many host animals undergoing natural or experimental trypanosome infections. Previous studies have shown that cattle infected with Trypanosoma congolense develop a pronounced thrombocytopenia (Maxie & Lossos, 1976), (Wellde, Kovatch, Chumo and Wykoff, 1978), (Forsberg, Valli, Gentry and Donworth, 1979). Thrombocytopenia was most severe during periods of high parasitemia and curative therapy of acutely infected animals induced a rapid thrombocytosis. In chronically infected animals which underwent intermittent parasitemia, there was an inverse relationship between levels of trypanosomes and thrombocytes. During periods of remission in parasitemia thrombocytes usually were found at normal or elevated levels. These findings indicated that an increased production and destruction of thrombocytes occurred in infected animals (Wellde et al. 1978). In an effort to clarify the etiology of the thrombocytopenia we determined the apparent survival times of <sup>51</sup>Cr labelled thrombocytes in normal steers and in steers acutely infected with Trypanosoma congolense.

MATERIALS AND METHODS

Animals

Ten steers of a predominant Charolais breed, ranging in age from 14 to 16 months and weighing between 170 to 200kg were used in these studies. The care and pre-experimental treatment of our animals has been previously described (Preston, Wellde and Kovatch, 1979). Infected and control animals were transferred to metabolism cages before transfusion with radioisotopically labelled platelets.

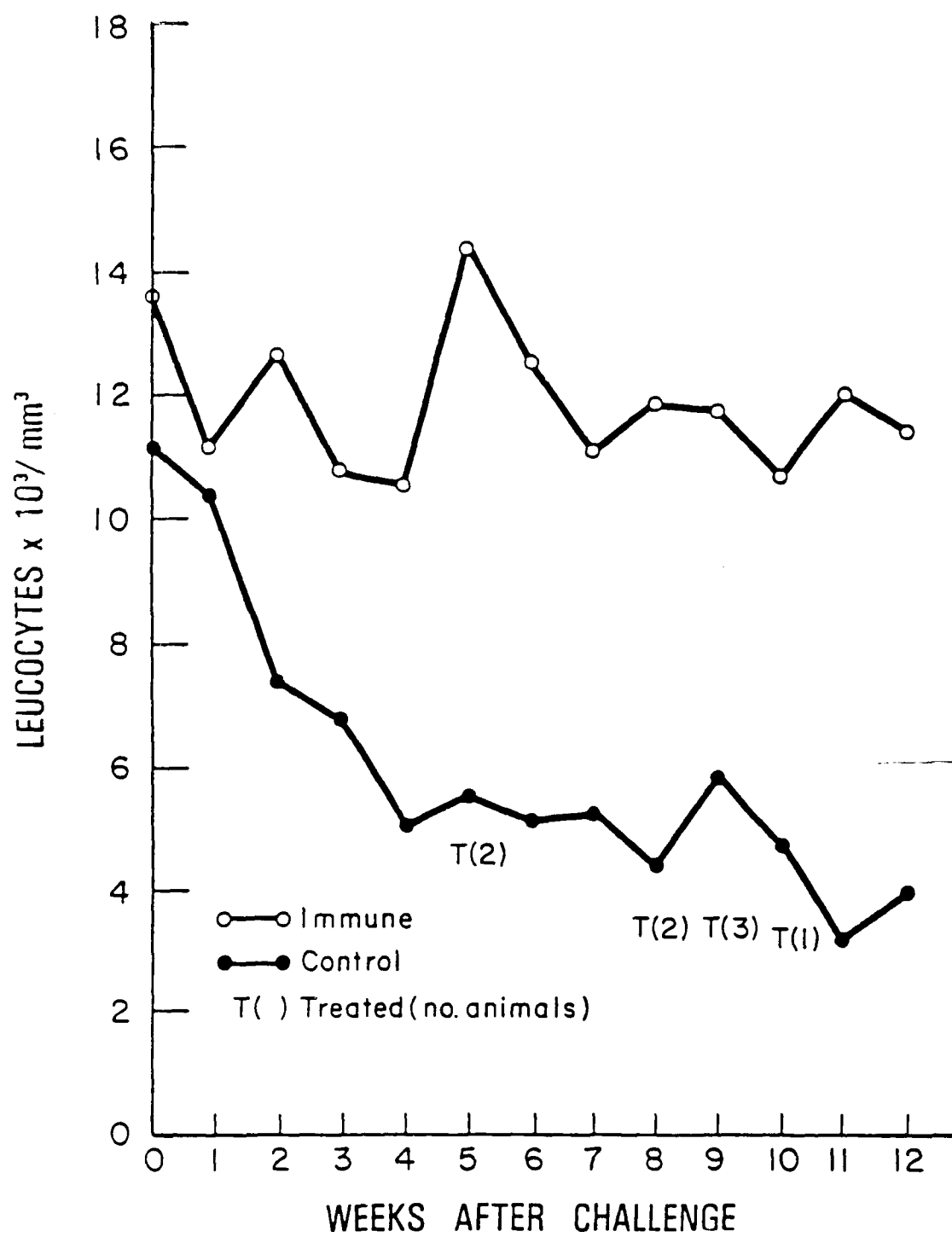
Parasites

The Trans Mara I strain of Trypanosoma congolense, the origin and maintenance of which is described elsewhere (Wellde, et al. 1974) was used to infect the experimental animals. Trypanosomes were collected from infected mice, counted in a hemocytometer and diluted with phosphate buffered saline (pH 7.8) containing 5% glucose and 10% fetal calf serum. Cattle were infected I.V. with  $1 \times 10^4$  T. congolense per 240kg.

#### LEGENDS FOR FIGURES

- FIGURE 1: The effect of numbers of Trypanosoma congolense injected on the prepatent period in bovines.
- FIGURE 2: Clinical parameters of young animals who survived Trypanosoma congolense infections. Data points where  $\pm$ SD overlap have not been plotted.
- FIGURE 3: An example of the stunting effect of Trypanosoma congolense infection on young animals can be seen in the animal in the foreground. The control is in the background. At the time of infection, 13 months previously, both animals were of similar age weight and stature.
- FIGURE 4: Clinical parameters of adult animals which either required treatment to survive or died from Trypanosoma congolense infection. Data points where  $\pm$ SD overlap have not been plotted.
- FIGURE 5: Parasitemias and packed cell volumes of an animal treated 82 days after primary Trypanosoma congolense infection and challenge 296 days later.
- FIGURE 6: Parasitemias and packed cell volumes of an immune and control animal challenged with  $1 \times 10^7$  Trypanosoma congolense from a relapse parasitemia obtained from a chronically infected bovine.
- FIGURE 7: Patterns of parasitemia in 5 immunized bovines after tsetse fly challenge with Trypanosoma congolense.
- FIGURE 8: Average parasitemia levels of immunized and control bovines after tsetse fly challenge with Trypanosoma congolense.
- FIGURE 9: Average packed cell volumes of immunized and control bovines after tsetse fly challenge with Trypanosoma congolense.
- FIGURE 10: Average thrombocyte levels of immunized and control bovines after tsetse fly challenge with Trypanosoma congolense.
- FIGURE 11: Average leucocyte levels of immunized and control bovines after tsetse fly challenge with Trypanosoma congolense.

Fig. 11





was much less pronounced but was consistent. We determined only the presence or absence of trypanosomes in the proboscis and midgut. Additional dissections are needed to compare numbers of trypanosome per infection in males with those of females. As expected, the overall colony infection rate (30.8%) was much higher than the 0.6 to 2.7% infection rates reported by Buxton (1955) for field collected G. morsitans.

It appeared that repeated feedings on an infected host after the teneral feeding increased fly infection rates. This is in contrast to the report by Clarke (1969) who concluded that G. morsitans could only be infected by T. congolense in initial feedings. While host parasitemia has not been directly related to fly infection, it seems likely that a threshold level of blood forms must be met for a cyclic infection to become established. The relatively high parasitemia of the host animal in our experiments and the fact that the Trans Mara strain has been maintained in an intense fly-bovine - fly cycle may contribute to the increased infection rate in flies exposed to multiple feedings.

Marked differences were apparent in the frequency of transmission to rats by flies known to be infected with T. congolense. Fairbairn and Burt (1946) found that infected G. morsitans transmitted T. rhodesiense to rats with 99.2% efficiency. Variation in host susceptibility probably could not account for the differences we observed in fly transmission of T. congolense since the non-transmitting flies engorged on 17 different rats, none of which became parasitemic (Table 3). Titration of Trans Mara strain metacyclics in rats and an estimate of the number of metacyclics transmitted by infected G. morsitans are needed. Still, it appears that individual flies differ in their ability to transmit the Trans Mara strain to rats.

An alternative possibility was suggested by Jenni and Coworkers (1979). They found that rat sera lyses immature T. brucei metacyclics. If this is true for T. congolense, consecutive feedings on rats may possibly reduce the number of metacyclics in the proboscis and ultimately affect the ability of the fly to transmit; however, 5 flies were able to transmit to rats at each of the 10 feedings.

It also seems possible that the observed differences in vector capacity are to some extent genetically influenced. Selection of progeny from infected flies for comparison with the main colony is still in progress. Selection for increased or decreased vector capacity has been successful in other arthropods, most notably mosquitoes. Continued interbreeding of infected flies is planned in an attempt to establish a genetic line of flies with increased infection and transmission rates. High colony infection rates are obviously desirable for future immunization attempts with metacyclics and for fly challenges of "immunized" or drug treated hosts.

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TABLE 1

Trypanosoma congolense (Trans Mara I)  
Infection in Randomly Selected Laboratory  
Reared Glossina morsitans Westw.\*

	No. dissected	No. Positive	
		Proboscis	Midgut
Females	118	32 (27.1%)	36 (30.5%)
Males	116	40 (34.5%)	46 (39.6%)
Total	234	72 (30.8%)	82 (35.0%)

\* Entire cages of 25-45-day old flies were sampled. The teneral feeding and 11 or more additional consecutive feedings were on a T. congolense infected host.

TABLE 2

Cyclic Trypanosoma congolense Infection in Groups of  
Glossina morsitans Westw. Exposed to Varying Numbers of  
Feedings on an Infected Bovine\*

No. Times Exposed	Proboscis Infection: No. Positive/No. Examined		
	Female	Male	Total
1	5/29 (17.2%)	4/15 (26.7%)	9/44 (20.5%)
2	5/22 (22.7%)	7/17 (41.2%)	12/39 (30.8%)
3	4/20 (20.0%)	5/17 (29.4%)	9/37 (24.3%)
4	7/23 (30.4%)	6/19 (31.6%)	13/42 (30.9%)
5	5/18 (27.8%)	5/17 (29.4%)	10/35 (28.6%)

\* Flies were fed on a non-infected host for a minimum of 18 consecutive feedings before dissection.

TABLE 3

Consecutive Feedings of Individual Trypanosoma congolense  
 Infected\* Glossina morsitans Westw. on Laboratory Rats

Fly No.	No. rats parasitemic/ No. exposed	Avg. days to parasitemia	Range in days
1	4/10 (40%)	20.3	16-23
2	1/10 (10%)	20.0	-
3	10/10 (100%)	18.0	14-22
4	0/7** (0%)	-	-
5	10/10 (100%)	15.2	10-18
6	9/10 (90%)	19.0	16-21
7	10/10 (100%)	17.8	15-21
8	0/10 (0%)	-	-
9	9/10 (90%)	20.3	17-24
10	10/10 (100%)	19.3	18-23
11	2/10 (20%)	21.5	21-22
12	10/10 (100%)	22.6	20-27
Totals	75/117 (64.1%)	19.4	10-27

\* All flies had transmitted prior detectable infections to individual rats.

\*\* Fly died after 7 feedings.

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